Genomed

JetQuick™ DNA Purification Kits

Gel Extraction, PCR Purification, and General DNA Clean-up

Cat. nos. 410050, 410250, 420050, 420250, 430050, and 430250

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User Manual

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Quantity	Cat. no.
JetQuick™ PCR Purification Spin Kit	50	410050
	250	410250
JetQuick™ Gel Extraction Spin Kit	50	420050
	250	420250
JetQuick™ General DNA Clean-up Kit	50	430050
	250	430250

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

JetQuick™ PCR Purification Spin Kit Contents

The components included in the JetQuick™ PCR Purification Spin Kit are listed below. **Store all components at room temperature.**

Component	Cat. No. (Quantity)	
	410050 (50 preps)	**410250 (250 preps)**
Binding Buffer (H1)	22 mL	110 mL
Wash Buffer (H2)	12.8 mL	60 mL
TE Buffer	10 mL	22 mL
Spin Columns	50 each	250 each
Receiver Tubes	50 each	250 each

Continued on next page

Kit Contents and Storage, Continued

JetQuick™ Gel Extraction Spin Kit Contents

The components included in the JetQuick™ Gel Extraction Spin Kit are listed below. **Store all components at room temperature.**

Component	Cat. No. (Quantity)	
	420050 (50 preps)	**420250 (250 preps)**
Gel Solubilization Buffer (L1)	100 mL	500 mL
Wash Buffer (L2)	12.8 mL	60 mL
TE Buffer	10 mL	22 mL
Spin Columns	50 each	250 each
Receiver Tubes	50 each	250 each

JetQuick™ General DNA Clean-up Kit Contents

The components included in the JetQuick™ General DNA Clean-up Kit are listed below. **Store all components at room temperature.**

Component	Cat. No. (Quantity)	
	430050 (50 preps)	**430250 (250 preps)**
Binding Buffer (M1)	22 mL	110 mL
Wash Buffer (M2)	12.8 mL	60 mL
TE Buffer	10 mL	22 mL
Spin Columns	50 each	250 each
Receiver Tubes	50 each	250 each

Continued on next page

Kit Contents and Storage, Continued

Buffer Composition

The composition of buffers included in the JetQuick™ Kits is listed below.

Kit	Buffer	Composition
JetQuick™ PCR Purification Spin Kit	Binding Buffer (H1)	When reconstituted, contains Tris-HCl, guanidine hydrochloride, isopropanol and EDTA
	Wash Buffer (H2)	When reconstituted, contains Tris-HCl, ethanol, NaCl, and EDTA
JetQuick™ Gel Extraction Spin Kit	Gel Solubilization Buffer (L1)	Contains guanidine isothiocyanate
	Wash Buffer (L2)	When reconstituted, contains Tris-HCl, ethanol, NaCl, and EDTA
JetQuick™ General DNA Clean-up Kit	Binding Buffer (M1)	When reconstituted, contains Tris-HCl, guanidine hydrochloride, isopropanol and EDTA
	Wash Buffer (M2)	When reconstituted, contains Tris-HCl, ethanol, NaCl, and EDTA

Kit Specifications

JetQuick™ PCR Purification Spin Kit

The specifications for the JetQuick™ PCR Purification Spin Kit are listed below.

Time required	5 minutes
Column Reservoir Capacity	600 µL
Centrifuge Compatibility	>12,000 × *g*
Elution Volume	50 µL
DNA Yield	Up to 20 µg
DNA Recovery	Up to 95% (depending on DNA fragment size)
DNA Fragment Size	80 bp to 20 kb
Primer Removal	>99.8%

JetQuick™ Gel Extraction Spin Kit

The specifications for the JetQuick™ Gel Extraction Spin Kit are listed below.

Time required	25 minutes
Starting Material	≤400 mg agarose gel slice
Binding Capacity	~20 µg DNA per column
Column Reservoir Capacity	600 µL
Centrifuge Compatibility	>12,000 × *g*
Elution Volume	50 µL
DNA Recovery	Up to 95% (depending on DNA fragment size)
DNA Fragment Size	40 bp to 10 kb

Continued on next page

Kit Specifications, Continued

JetQuick™ General DNA Clean-up Kit

The specifications for the JetQuick™ General DNA Clean-up Kit are listed below.

Time required	5 minutes
Column Reservoir Capacity	600 µL
Centrifuge Compatibility	>12,000 × *g*
Elution Volume	50 µL
DNA Yield	Up to 20 µg
DNA Recovery	Up to 95% (depending on DNA fragment size)
DNA Fragment Size	80 bp to 20 kb
Enzyme removal (e.g., T4 DNA ligase, *Taq* DNA polymerase, restriction enzymes)	no detectable activity

PCR Purification

System Overview

About the Kit

The JetQuick™ PCR Purification Spin Kit provides a simple and reliable method to isolate highly pure DNA on the basis of spin columns. It is designed for the rapid and efficient removal of primers, dNTPs, enzymes, and salts from PCR products. The JetQuick™ PCR Purification Spin Kit is based on the selective binding of dsDNA to a silica-based membrane in the presence of chaotropic salts.

The PCR product is mixed with Binding Buffer (H1) to adjust conditions for subsequent dsDNA binding to the JetQuick™ Spin Column. The dsDNA binds to the silica-based membrane in the column and impurities are removed by thorough washing with Wash Buffer (H2). The dsDNA is then eluted in low salt Elution Buffer (Tris-HCl or TE) or water.

Downstream Applications

DNA purified using the JetQuick™ PCR Purification Spin Kit is as pure as CsCl purified DNA and is suitable for a number of downstream applications including:

- Ligation
- Restriction Analysis
- Automated and manual DNA sequencing
- PCR amplification
- Cloning

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Advantages

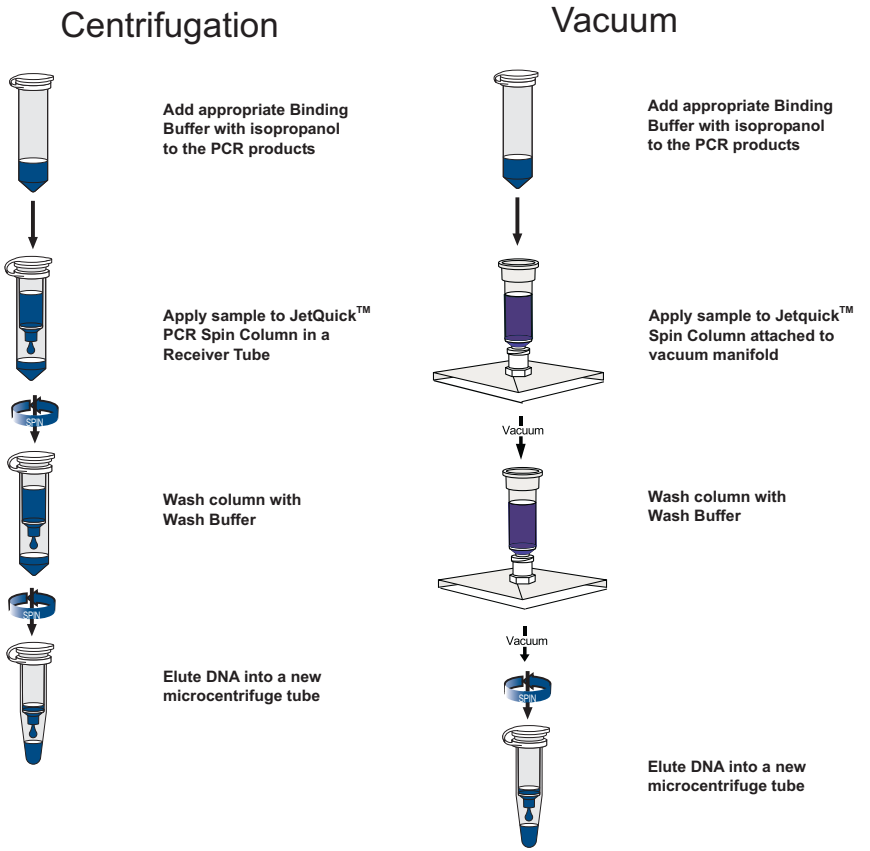
The advantages of using the JetQuick™ PCR Purification Spin Kit are:

- Efficient removal of primers, dNTPs, salts, and enzymes without the need to perform ethanol precipitation or use solvents such as phenol or chloroform
- Reliable performance of the purified PCR products in downstream applications
- Not sensitive to gelatin or detergents such as NP-40 or Triton X-100 (at concentrations <0.1%)
- May be used to isolate dsDNA fragments ≥100 bp

Experimental Overview

Introduction

The flow chart below provides an overview for purifying DNA from Polymerase Chain Reactions using the JetQuick™ PCR Purification Spin Kit.


Centrifugation
Vacuum
Add appropriate Binding Buffer with isopropanol to the PCR products
Add appropriate Binding Buffer with isopropanol to the PCR products
Apply sample to JetQuick™ PCR Spin Column in a Receiver Tube
Apply sample to Jetquick™ Spin Column attached to vacuum manifold
SPIN
Vacuum
Wash column with Wash Buffer
Wash column with Wash Buffer
SPIN
Vacuum
SPIN
Elute DNA into a new microcentrifuge tube
Elute DNA into a new microcentrifuge tube

PCR Purification Procedure

Introduction

The PCR purification procedure is designed for purifying up to 20 µg dsDNA using a centrifuge (see page 5) or vacuum manifold (see page 6) in ~5 minutes.

Materials Supplied by the User

- Isopropanol
- 96–100% ethanol
- Microcentrifuge capable of centrifuging at >12,000 × *g*
- Vacuum manifold such as the EveryPrep™ Universal Vacuum Manifold (for **Purification Procedure Using Vacuum** only, see page 27 for ordering information)
- DNase-free pipettes and tips

Before Starting

- Reconstitute **Binding Buffer (H1)** with isopropanol according to the instructions on the label of the bottle. Store reconstituted Binding Buffer (H1) at room temperature.
- Reconstitute **Wash Buffer (H2)** with absolute ethanol (96–100%) according to the instructions on the label of the bottle. Store reconstituted Wash Buffer (H2) at room temperature.

Method for Purifying DNA

- To purify DNA using centrifugation, proceed to page 5.
- To purify DNA using vacuum, proceed to page 6.

Continued on next page

PCR Purification Procedure, Continued

Purification Procedure Using Centrifugation

1. Add 4 volumes of Binding Buffer (H1) to 1 volume of PCR sample. Mix well.

   Note: When PCR assays >100 µL are used, scale up Buffer H1 proportionally and maintain the 4:1 ratio of Buffer H1:PCR Sample. In this case, multiple loadings of the JetQuick™ Spin Column (Step 3, below) are required.
2. Place a JetQuick™ Spin Column into a 2 mL Receiver Tube.
3. Load the mixture from Step 1 into the prepared column.
4. Centrifuge at >12,000 × *g* for 1 minute.
5. Re-insert the column into the empty receiver tube and add 500 µL of reconstituted Wash Buffer (H2).
6. Centrifuge the column at >12,000 × *g* for 1 minute.
7. Discard the flow-through and place the JetQuick™ Spin Column back in the same 2 mL Receiver Tube.
8. Centrifuge again at >12,000 × *g* for 1 minute.

   Note: The additional centrifugation ensures that no residual ethanol, which may interfere with downstream applications, is carried over into the next step of the protocol. Buffer H2 is not completely removed unless the flow-through is discarded before centrifugation.
9. Place the JetQuick™ Spin Column into a new 1.5 mL microcentrifuge tube (not supplied in the kit).
10. Add 50 µL of sterile water, TE Buffer, or 10 mM Tris-HCl, pH 8.0 to the center of the column.
11. Centrifuge at >12,000 × *g* for 2 minutes.

    The elution tube contains your purified PCR product. Remove and discard the column.

    Note: To obtain higher DNA concentrations, elute with 30 µL preheated (65°C to 70°C) elution buffer. Add the buffer directly onto the center of the silica membrane of the JetQuick™ Spin Column and let stand for 1 minute before centrifugation. Preheated elution buffer is recommended to elute PCR fragments >5kb.

Continued on next page

PCR Purification Procedure, Continued

Purification Procedure Using Vacuum

Perform all vacuum operations at room temperature.

1. Add 4 volumes of Binding Buffer (H1) to 1 volume of amplification reaction and mix thoroughly.

   Note: When PCR assays >100 µL are used, scale up Buffer H1 proportionally, maintaining the 4:1 ratio of Binding Buffer:PCR Sample. In this case, multiple successive loadings of the column (Step 3, below) are required.

2. Assemble the vacuum manifold according to the manufacturer's instructions and attach a JetQuick™ Spin Column to the vacuum manifold.
3. Pipette the DNA sample (Step 1, above) onto the center of the silica membrane of the JetQuick™ Spin Column.
4. Apply vacuum (−200 to −650 mbar) until all liquid passes through the column and then turn off the vacuum source.
5. Add 500 µL Wash Buffer (H2) containing ethanol (page 4).
6. Apply vacuum as described in Step 4. Remove the column from the vacuum and place it into a 2 mL Receiver Tube.
7. Centrifuge the column with the Receiver Tube at maximum speed for 1 minute to remove any residual Wash Buffer (H2) and ethanol. Discard the flow-through and the Receiver Tube.
8. Place the JetQuick™ Spin Column into a new 1.5 mL microcentrifuge tube (not supplied in the kit) and add 50 µL of sterile water, TE Buffer, or 10 mM Tris-HCl, pH 8.0 to the center of the column.
9. Centrifuge at >12,000 × *g* for 2 minutes. *The elution tube contains your purified PCR product.* Discard the column.

   Note: A higher DNA concentration is obtained if the elution is performed with only 30 µL of elution buffer. In this case, preheat the elution buffer to 65°C–70°C, add the buffer directly onto the center of the spin column and let stand for 1 minute before centrifugation.

Continued on next page

PCR Purification Procedure, Continued

Storing DNA

- Store purified DNA at 4°C for immediate use.
- Aliquot DNA and store at –20°C for long-term storage.
- Store DNA eluted in water at –20°C.
- Avoid repeated freezing and thawing of DNA.

Troubleshooting

Introduction Review the information below to troubleshoot your experiments with the JetQuick™ PCR Purification Spin Kit.

Problem	Cause	Solution
Low DNA yield	Insufficient amplification	Check amplicon on gel to verify the PCR product prior to purification.
	Incorrect binding conditions	For efficient DNA binding, always mix 1 volume of PCR assay with 4 volumes of Binding Buffer (H1).
	Incomplete DNA elution	Add preheated Elution Buffer (65°C to 70°C) directly onto the center of the silica matrix of the spin column and let it stand for 1 minute. Centrifuge at >12,000 × *g* for 1 minute.
	DNA fragment is too large	Use preheated Elution Buffer (as described above).
Inhibition of enzymatic reaction	Residual ethanol in the purified DNA	Traces of ethanol from the Wash Buffer (H2) can inhibit downstream enzymatic reactions. To remove Wash Buffer (H2), discard the Wash Buffer flow-through from the Receiver Tube. Place the column into the Receiver Tube and centrifuge the column at >12,000 × *g* for 2–3 minutes to completely dry the column.
	Residual salt in the purified DNA	Use water or 10 mM Tris-HCl, pH 8.0 (no EDTA) for elution.

Gel Extraction

System Overview

About the Kit

The JetQuick™ Gel Extraction Spin Kit is used for the rapid and efficient purification of DNA fragments from TAE or TBE agarose gels. DNA fragments up to 10 kb are optimally purified, although DNA fragments >10 kb may be purified with lower DNA recovery. Extract DNA by first excising and dissolving the gel (pages 12–13) and then purify the DNA by centrifugation (pages 14–15) or vacuum (see pages 15–16) using JetQuick™ silica membrane-based columns.

Note: The JetQuick™ Gel Extraction Spin Kit is **not** designed to purify supercoiled plasmid DNA or genomic DNA from agarose gels. **Only linear DNA fragments are purified from gels using this kit.**

Advantages

Advantages of using the JetQuick™ Gel Extraction Spin Kit are:

- Purification of DNA fragments from TAE and TBE agarose gels of various percentages and melting points
- Procedure is complete in less than 25 minutes
- Efficient purification of DNA fragments from 40 bp to 10 kb from gels
- High recovery (85–95%) of DNA fragments
- High binding column capacity of up to 20 µg DNA

Note

- Ensure that the DNA fragment of interest is **completely** separated from other DNA fragments on the agarose gel.
- Each JetQuick™ Spin Column can purify **up to 20 µg** of DNA. If you wish to purify a larger amount of DNA, use several JetQuick™ Spin Columns. For best results, use one JetQuick™ Spin Column per 10 µg of DNA fragment loaded onto the gel.

Continued on next page

System Overview, Continued

Follow the recommendations below to obtain the best results:

- To minimize DNA degradation, always wear gloves and use a clean razor blade to cut the gel slice.
- Maintain a sterile working environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the sterile buffers supplied with the kit.
- Make sure all equipment that comes in contact with DNA is sterile, including pipette tips and tubes.

Experimental Summary

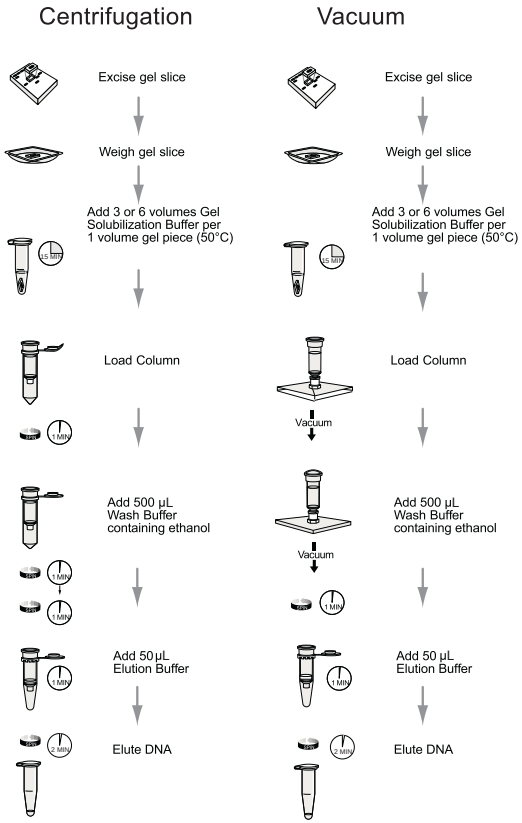
To purify DNA fragments from agarose gels using the JetQuick™ Gel Extraction Spin Kit:

1. Dissolve an excised gel piece using Gel Solubilization Buffer (L1).
2. Purify and elute the DNA fragment using centrifugation or vacuum.
3. Place the dissolved gel piece into a JetQuick™ Spin Column containing a silica membrane and allow the DNA to bind the membrane.
4. Wash the membrane with Wash Buffer (L2) containing ethanol to remove impurities.
5. Elute the purified DNA using Elution Buffer (TE Buffer). The purified DNA is suitable for use in a wide variety of downstream applications.

Experimental Overview

Introduction

The flow chart below provides an overview for purifying DNA fragments from agarose gels using the JetQuick™ Gel Extraction Kit. First, excise and dissolve the gel (pages 12–13). To purify DNA using centrifugation, see page 14. To purify DNA using vacuum, see page 15.



Gel Extraction Procedure

Introduction

The Gel Extraction procedure described on pages 12–16 is designed for purifying up to 20 µg dsDNA using centrifugation or a vacuum manifold in ~25 minutes.

Materials Supplied by the User

- 96–100% ethanol
- Agarose gel containing the DNA fragment
- Weighing paper or weigh trays
- Scale (sensitive to 0.001 g)
- Water bath or heat block set at 50°C
- 1.5 mL or 5 mL polypropylene microcentrifuge tubes
- Clean, sharp razor blade
- Vacuum manifold such as the EveryPrep™ Universal Vacuum Manifold (for **Purification Using Vacuum**, only, see page 27 for ordering information)

Excising the gel

After completing agarose gel electrophoresis:

1. Excise the area of the gel containing your desired DNA fragment using a clean, sharp razor blade. Minimize the amount of agarose surrounding the DNA fragment.
2. Weigh the gel slice containing the DNA fragment using a scale sensitive to 0.001 g, and then place the gel into a 1.5 or 5.0 mL microcentrifuge tube as described on the next page.

   Note: The maximum amount of starting material (gel) is 400 mg per tube. If your gel slice exceeds 400 mg, cut the gel into smaller slices so that no one piece exceeds 400 mg. Place each additional gel slice into separate microcentrifuge tubes. For highest DNA purity, use 100 mg agarose gel slices per extraction.
3. Proceed to **Dissolving the Gel**, next page.

Continued on next page

Gel Extraction Procedure, Continued

Gel Solubilization Buffer (L1) contains guanidine isothiocyanate. Use proper precautions when handling.

Dissolving the Gel

1. **For ≤2% agarose gels:**
   - Place up to 400 mg of the excised gel containing the DNA fragment (above) into a **1.5 mL** polypropylene microcentrifuge tube.
   - Add 3 volumes Gel Solubilization Buffer (L1) for every 1 volume of gel (*e.g.,* add 1.2 mL Buffer L1 for a 400 mg gel piece).

   For >2% agarose gels:
   - Place up to 400 mg of the excised gel containing the DNA fragment (see above) into a **5 mL** polypropylene microcentrifuge tube.
   - Add 6 volumes Gel Solubilization Buffer (L1) for every 1 volume of gel (*e.g.*, add 2.4 mL Buffer L1 for a 400 mg gel piece).
2. Place the tube(s) containing your gel slice and Buffer L1 (Step 1) into a 50°C water bath or heat block.
3. Incubate at 50°C for 15 minutes. Invert the tube by hand every 3 minutes to mix and ensure gel dissolution.

   Note: Complete dissolution of the agarose gel slice is critical. Once the gel slice appears dissolved, incubate the gel slice for an additional 5 minutes at 50°C. High concentration gels (>2% agarose) or large gel slices may take 20–30 minutes to dissolve. Cutting larger gel slices into smaller pieces will enhance solubilization.
4. Proceed to **Purification Using Centrifugation,** next page or **Purification Using Vacuum,** page 15.

Continued on next page

Gel Extraction Procedure, Continued

Purification Using Centrifugation

The bottle of Buffer L2 contains concentrated buffer solution. **Before beginning**, add absolute ethanol (96–100%) as directed on the label of the bottle. Store reconstituted Wash Buffer (L2) at room temperature.

1. Place a JetQuick™ Spin Column into a 2 mL Receiver Tube. Pipette the dissolved gel piece containing the DNA fragment of interest (Step 3, previous page) onto the JetQuick™ Spin Column.

   Note: Do **not** load >400 mg agarose per JetQuick™ Spin Column. The maximum column capacity is 600 µL; for larger sample volumes, multiple loadings are necessary.

2. Centrifuge at >12,000 × *g* for 1 minute. Discard the flow-through and replace the JetQuick™ Spin Column into the 2 mL Receiver Tube.

   Note: For downstream applications including sequencing, *in vitro* transcription, or microinjection, or if the weight of the initial agarose gel slice is >250 mg, wash the DNA with 500 µL Buffer L1. Incubate for 1 minute at room temperature before centrifuging again at >12,000 × *g* for 1 minute.

3. Add 500 µL Wash Buffer (L2), containing ethanol to the JetQuick™ Spin Column.

4. Centrifuge at >12,000 × *g* for 1 minute. Discard the flow-through and replace the column into the Receiver Tube.

5. Centrifuge again at maximum speed for 1 minute to remove any residual Wash Buffer (L2) and ethanol.

   Note: Discard the flow-through before centrifugation to remove residual Wash Buffer (L2). The additional centrifugation step assures that no residual ethanol is carried over into the next step.

6. Discard the Receiver Tube and place the JetQuick™ Spin Column into a clean 1.5 mL microcentrifuge tube.

Continued on next page

Gel Extraction Procedure, Continued

Purification Using Centrifugation Continued

7. Add 50 µL of sterile water, 10 mM Tris-HCl, pH 8.0, or TE Buffer to the center of the JetQuick™ Spin Column.
8. Centrifuge at >12,000 × *g* for 2 minutes. The tube contains the purified DNA. Discard the column.

   Note: To obtain higher DNA concentrations, elute with only 30 µL of preheated (65°C to 70°C) elution buffer, dispensed directly onto the center of the membrane. In this case, let stand for 1 minute before centrifugation.
9. Store DNA as indicated on page 16.

Purification Using Vacuum

Before beginning, add ethanol to the Wash Buffer (L2).

Perform all vacuum operations at room temperature.

1. Assemble the vacuum manifold according to the manufacturer's instructions.
2. Attach a JetQuick™ Spin Column to the vacuum manifold.
3. Pipette the dissolved gel piece containing the DNA fragment of interest (Step 3, page 13) onto the center of the silica membrane of the JetQuick™ Spin Column.
4. Apply vacuum (−200 to −650 mbar) until all liquid passes through the column, and then switch off the vacuum source.

   Note: Do not load more than 400 mg agarose per column.
5. *Optional Column Wash*: Add 500 µL Gel Solubilization Buffer (L1) to the center of the JetQuick™ Spin Column. Let stand for 1 minute. Apply vacuum (−200 to −650 mbar) until all liquid passes through the column, and then switch off the vacuum source.

Continued on next page

Gel Extraction Procedure, Continued

Purification Using Vacuum, Continued

6. Add 500 µL Wash Buffer (L2) containing ethanol to the center of the JetQuick™ Spin Column.
7. Apply vacuum (–200 to –650 mbar) until all liquid passes through the column, and then switch off the vacuum source.
8. Remove the JetQuick™ Spin Column from the vacuum and place it into a 2 mL Receiver Tube.
9. Centrifuge the column with the Receiver Tube at maximum speed for 1 minute to remove any residual Wash Buffer and ethanol. Discard the Receiver Tube with the flow-through.
10. Place the JetQuick™ Spin Column into a clean 1.5 mL recovery tube.
11. Add 50 µL of sterile water (or TE Buffer or 10 mM Tris-HCl, pH 8.0) to the center of the JetQuick™ Spin Column.

    Note: Higher DNA concentrations are obtained if the elution is performed with only 30 µL of elution buffer preheated to 65°C –70°C. Add the buffer onto the center of the silica matrix of the spin column.
12. Incubate for 1 minute at room temperature.
13. Centrifuge at >12,000 × *g* for 2 minutes to elute the purified DNA *into the recovery tube.* Discard the JetQuick™ Spin Column.

Storing DNA

- Store the purified DNA at 4°C for immediate use.
- Aliquot the DNA and store at –20°C for long-term storage.
- Store DNA eluted in water at –20°C.
- Avoid repeated freezing and thawing of DNA.

Troubleshooting

Introduction Review the information below to troubleshoot your experiments with the JetQuick™ Gel Extraction Spin Kit.

Problem	Cause	Solution
Low DNA yield	Incorrect ratio of gel to Gel Solubilization Buffer (L1)	Ensure that the correct volume of Gel Solubilization Buffer (L1) is added for every 1 volume of gel used, based on the agarose gel percentage (see page 13).
	Incomplete solubilization of gel piece	Verify that the temperature of water bath or heat block is at 50°C. Cut large gel slices into several pieces to accelerate gel dissolution. Mix gel slice in Buffer L1 every 3 minutes during the dissolution step.
	Incomplete DNA elution	Preheat Elution Buffer to 65°C to 70°C, add the buffer directly onto the center of the silica matrix of the JetQuick™ Spin Column, let stand for 1 minute. Centrifuge at >12,000 × *g* for 1 minute.
	DNA fragment is too large	Use preheated elution buffer (as described above). Increase the incubation time for elution to >10 minutes.
	DNA is supercoiled	This kit is not designed to purify supercoiled plasmid DNA.
Inhibition of enzymatic reaction	Residual ethanol in the purified DNA	Traces of ethanol from the Wash Buffer (L2) can inhibit downstream enzymatic reactions. To remove Wash Buffer (L2), discard Buffer L2 flow-through from the Receiver Tube. Place the column into the Receiver Tube and centrifuge at >12,000 × *g* for 3 minutes to completely dry the column.
	Residual salt in the purified DNA	Use water or 10 mM Tris-HCl, pH 8.0 (no EDTA) for elution.

General DNA Clean-Up

System Overview

About the Kit

The JetQuick™ General DNA Clean-up Kit is used to purify DNA in less than 5 minutes. The DNA sample (e.g., crude plasmid preps, restriction, ligation or other enzymatic assay) is first adjusted to defined salt conditions. The DNA selectively binds to the silica membrane of a JetQuick™ Spin Column during centrifugation. After centrifugation, one wash is sufficient to remove nucleotides, enzymes, salts, dyes, residual RNA's and other impurities. The purified DNA is eluted in TE Buffer or water and ready for downstream use.

DNA can be purified by centrifugation (page 21) or vacuum (see page 22) using JetQuick™ silica membrane-based columns.

Advantages

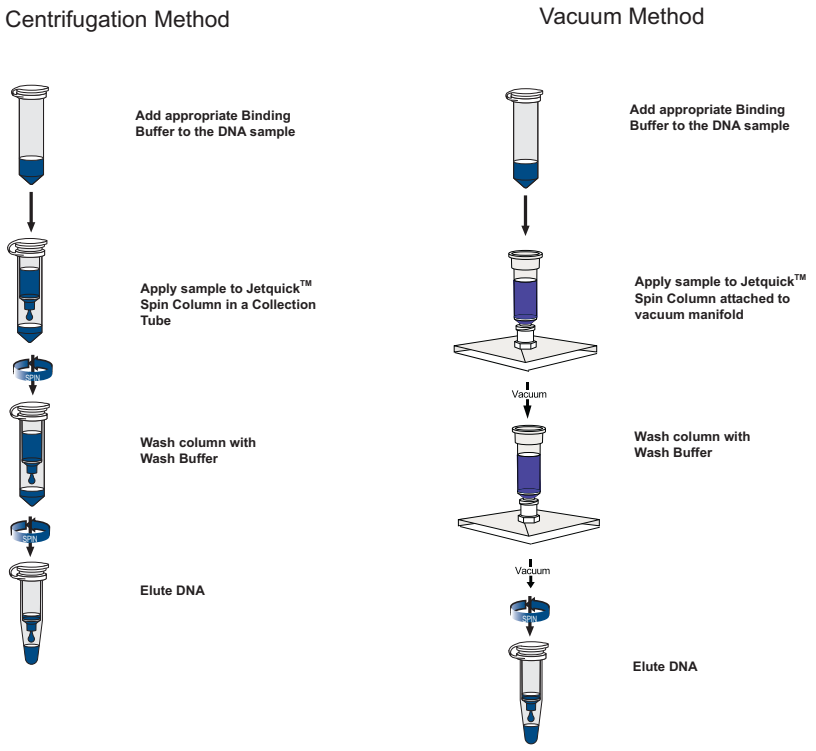
Advantages of using JetQuick™ General DNA Clean-up Kits are:

- No phenol
- No chloroform
- Total salt, dye, and protein removal
- Not sensitive to gelatin or detergents such as Triton X-100 (at concentrations <0.1%) or NP-40

Experimental Overview

Introduction

The flow chart below provides an overview for purifying DNA using the JetQuick™ General DNA Clean-up Kit. Purification may be performed using centrifugation or vacuum.



General DNA Clean-Up Procedure

Introduction

The General DNA Clean-Up procedure described on pages 21–23 is designed for purifying up to 20 µg dsDNA using a centrifuge or vacuum manifold in ~5 minutes.

Materials Supplied by the User

- Isopropanol
- 96–100% ethanol
- Microcentrifuge capable of centrifuging at >12,000 × *g*
- DNase-free pipettes and tips
- DNA sample from enzymatic and or other *in vitro* assays (e.g., restriction enzyme digestion, ligation reaction) or plasmid DNA from crude minipreps
- Vacuum manifold such as the EveryPrep™ Universal Vacuum Manifold, for **Purification Procedure Using Vacuum** only (see page 27 for ordering information)

Before Starting

Reconstitute **Binding Buffer (M1)** with isopropanol and **Wash Buffer (M2)** with absolute ethanol (96–100%) according to the instructions on the labels of the bottles. Store reconstituted Binding Buffer (M1) and Wash Buffer (M2) at room temperature.

Reconstituted Binding Buffer (M1) contains guanidine hydrochloride and isopropanol. Always wear a lab coat, disposable gloves, and protective eyewear during handling.

Do not add bleach or acidic solutions directly to solutions containing guanidine hydrochloride or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

Continued on next page

General DNA Clean-Up Procedure, Continued

Purification Procedure Using Centrifugation

1. Add 4 volumes of Binding Buffer (M1) to 1 volume of DNA sample. Mix well.

   Note: When samples >100 µL are used**,** scale up the volume of Buffer M1 proportionally, maintaining the ratio of Buffer M1:DNA sample (4:1). In this case, multiple loadings of the column (Step 3, below), are required.
2. Place a JetQuick™ Spin Column into a 2 mL Receiver Tube.
3. Load the mixture from Step 1 into the prepared column.
4. Centrifuge at >12,000 × *g* for 1 minute.
5. Discard the flow-through.
6. Re-insert the column into the empty Receiver Tube and add 500 µL of reconstituted Wash Buffer (M2).
7. Centrifuge the column at >12,000 × *g* for 1 minute.
8. Discard the flow-through and place the JetQuick™ Spin Column back in the same Receiver Tube.
9. Centrifuge again at >12,000 × *g* for 1 minute.

   Note: Residual buffer (M2) will not be completely removed unless the flow-through is discarded before this additional centrifugation. Buffer M2 contains ethanol, and residual ethanol may interfere with subsequent reactions. The additional centrifugation assures that no residual ethanol is carried over into the next step of the protocol.
10. Place the JetQuick™ Spin Column into a new 1.5 mL microcentrifuge tube.
11. Add 50 µL of sterile water, TE Buffer, or 10 mM Tris-HCl, pH 8.0, to the center of the column.
12. Centrifuge at >12,000 × *g* for 2 minutes.

    Note: A higher DNA concentration is obtained if the elution is performed with only 30 µL of elution buffer. In this case, preheat the elution buffer to 65°C–70°C, add the buffer directly onto the center of the spin column and let stand for 1 minute before centrifugation.

Continued on next page

General DNA Clean-Up Procedure, Continued

Purification Procedure Using Vacuum

1. Add 4 volumes of Binding Buffer (M1) to 1 volume of DNA sample. Mix well.

   Note: When samples >100 µL are used**,** scale up the volume of Buffer M1 proportionally, maintaining the ratio of Buffer M1:DNA sample (4:1). In this case, multiple loadings of the column (Step 4, below), are required.
2. Assemble the vacuum manifold according to the manufacturer's instructions.
3. Attach a column to the vacuum manifold.
4. Pipette the sample onto the membrane of the column. Apply vacuum (−200 to −650 mbar) until all liquid passes through the column, and then switch off the vacuum source.
5. Add 500 µL Wash Buffer (M2) containing ethanol (see page 20) to the center of the JetQuick™ Spin Column.
6. Apply vacuum (−200 to −650 mbar) until all liquid passes through the column, and then switch off the vacuum. Remove the JetQuick™ Spin Column from the vacuum and place it into a 2 mL Receiver Tube.
7. Centrifuge the column with the Receiver Tube at maximum speed for 1 minute to remove any residual Wash Buffer (M2) and ethanol. Discard the flow-through and the Receiver Tube.
8. Place the JetQuick™ Spin Column into a new 1.5 mL microcentrifuge tube. Add 50 µL of sterile water, TE Buffer, or 10 mM Tris-HCl, pH 8.0, to the center of the column.
9. Centrifuge at >12,000 × *g* for 2 minutes.

   Note: A higher DNA concentration is obtained if the elution is performed with only 30 µL of elution buffer. In this case, preheat the elution buffer to 65°C–70°C, add the buffer directly onto the center of the spin column and let stand for 1 minute before centrifugation.

Continued on next page

General DNA Clean-Up Procedure, Continued

Storing DNA

- Store the purified DNA at 4°C for immediate use.
- Aliquot the DNA and store at –20°C for long-term storage.
- Store DNA eluted in water at –20°C.
- Avoid repeated freezing and thawing of DNA.

Troubleshooting

Introduction Review the information below to troubleshoot your experiments with the JetQuick™ General DNA Clean-up Kit.

Problem	Cause	Solution
Low DNA yield	Insufficient starting material	Verify the quantity of DNA in your sample prior to purification.
	Incorrect binding conditions	For efficient DNA binding, always mix 1 volume of DNA sample with 4 volumes of Binding Buffer (M1).
	Incomplete DNA elution	Add preheated Elution Buffer (65°C to 70°C) directly onto the center of the silica matrix of the spin column and let stand for 1 minute. Centrifuge at >12,000 × *g* for 1 minute.
	DNA fragment is too large	Use preheated Elution Buffer for all fragments >7.5kb and add the buffer directly onto the center of the silica matrix of the spin column. Let stand for 1 minute before centrifuging at >12,000 × *g* for 1 minute.
Inhibition of enzymatic reaction	Residual ethanol in the purified DNA	Traces of ethanol from the Wash Buffer (M2) can inhibit downstream enzymatic reactions. To remove Buffer M2, discard Wash Buffer flow-through from the Receiver Tube. Place the column into the Receiver Tube and centrifuge the column at >12,000 × *g* for 2–3 minutes to completely dry the column.
	Residual salt in the purified DNA	Use water or 10 mM Tris-HCl, pH 8.0 (no EDTA) for elution.

Appendix

Estimating DNA Yield and Quality

DNA Yield

Perform DNA quantitation using Quant-iT™ DNA Assay Kits, agarose gel electrophoresis, or spectrophotometer.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page 27 for ordering information) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), and other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. For optimal results, perform the quantitation using Invitrogen's Qubit® Fluorometer (page 27). Quant-iT™ DNA Assay Kits also work with standard fluorescent microplate readers. Follow the manufacturer's recommendations to perform the assay.

Agarose Gel Electrophoresis

To estimate the yield using agarose gel electrophoresis, compare the purified PCR product to known quantities of a DNA fragment with the same size. Compare the band intensity of the purified PCR product to the DNA fragment used as a standard.

Continued on next page

Estimating DNA Yield and Quality, Continued

DNA Yield, Continued

UV Spectrophotometer

To estimate DNA yield by spectrophotometer, measure the UV absorbance of the sample at 260 nm. Dilute the sample in Tris-HCl pH 7.5–8.5. Calculate quantity with the following formula:

$$A_{260} \times \text{dilution factor} \times \varepsilon$$

Where A_{260} is the absorbance at 260 nm, and ε is the molar extinction coefficient (for dsDNA, ε = 50; for, ssDNA ε = 33).

Estimating DNA Quality

DNA purified using JetQuick™ DNA Purification Kits has an OD A_{260}/A_{280} value of >1.8 when samples are diluted in Tris-HCl pH 7.5–8.5 indicating that the DNA is substantially free of contaminants that would otherwise affect UV absorbance.

To confirm the integrity and the size of the purified DNA fragments, perform agarose gel electrophoresis.

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with JetQuick™ DNA Purification Kits.

For more information about these products, visit www.invitrogen.com or contact Technical Support (page 28).

Product	Amount	Cat. no.
Quant-iT™ DNA Assay Kit, High Sensitivity	1,000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad–Range	1,000 assays	Q33130
Qubit® Fluorometer	1 each	Q32857
EveryPrep™ Universal Vacuum Manifold	1 unit	K211101

Technical Support

World Wide Web

Visit the website at **www.genomed-dna.com** for:

- Technical resources, including manuals, MSDSs, FAQs
- Complete technical support contact information
- Access to the Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on **www.genomed-dna.com**.

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Purchaser Notification

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Purchaser Notification, Continued

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